







The purification and characterisation of hyaluronidase from the venom of the honey bee, Apis mellifera.

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Hyaluronidase has been purified from the venom of the honey bee, Apis mellifera. The purification proved remarkably difficult, requiring a large number of chromatographic steps culminating in the removal of traces of phospholipase A2 with an affinity purified rabbit anti-phospholipase A2 immunosorbent column. The purified enzyme showed a 1143-fold increase in specific activity and was homogeneous. Electrophoresis in polyacrylamide gels (12%) containing sodium dodecyl sulphate (pH 8.9) or urea (pH 2.8) and electrofocusing in polyacrylamide (5%) gave a single band. The final product contained less than 0.1% phospholipase A2 and less than 1.5% acid phosphatase and gave a single line of precipitation against rabbit anti-hyaluronidase but was not precipitated by rabbit anti-phospholipase A2. Previous reports of instability were not confirmed, and we found the enzyme to be highly stable over a wide range of temperature and pH, and to denaturing agents. Purified hyaluronidase was found to be 'sticky' when highly pure and at low concentration, and adhered strongly to Sephadex G-75. The relative molecular mass was estimated at 35 000-37 000 by gel filtration, and at 41 000 by sodium dodecyl sulphate/polyacrylamide gel electrophoresis. A value of 50 000 was obtained by ultracentrifugation assuming a partial specific volume of 0.73 cm<sup>3</sup>/g. Hyaluronidase was found to be a minor allergen in bee venom allergic patients.

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PMID: 6698011 [PubMed - indexed for MEDLINE]

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